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Preimplantational Ectogenesis

Science and Speculation Concerning *In Vitro* Fertilization and Related Procedures

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*In recent years, technical advances have made preimplantational ectogenesis (*in vitro *maturation, fertilization and early embryonic development) more than a theoretical concept. Such procedures hold great promise in medical research. However, despite our newly-acquired skills in tissue culture and microsurgical manipulation, and contrary to many sensational articles in the lay press, it is not likely that preimplantational ectogenesis will soon attain wide clinical use in humans. Adverse societal attitudes, based largely upon moral and ethical dilemmas, will probably combine with still-unresolved technical difficulties to restrict the clinical applications.*

PREIMPLANTATIONAL ECTOGENESIS is a concept charged with emotion. It has stimulated both hopes and fears, and the quantity of hysterical commentary spawned by it has been colossal. On the one hand we are told that if we truly love children, we will pray for "glass wombs"[1]; alternatively, this type of work has been described as representing "unethical experiments on the unborn."[2]

In reality, however, preimplantational ectogenesis is likely to neither save nor destroy civilization as we know it. Furthermore, its immediacy of application on anything resembling a wide scale is nowhere as near as many of its proponents and antagonists alike would have us believe. The field is very much in its infancy, but as it matures it is likely to present ever-increasing opportunities in both medical research and clinical practice. Associated with these opportunities will be numerous moral and ethical dilemmas, many of them entirely novel.

In this article, we will consider the present status and possible future applications of preimplantational ectogenesis, a broad term that may be defined as the development of a germ cell or an embryo outside the body of its mother during the time before it would normally implant in the uterus. For convenience, we will divide the discussion of ectogenesis in human and nonhuman species into three stages: culture of the unfertil-

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ABBREVIATIONS USED IN TEXT

DMSO = dimethysulfoxide
DNA = deoxyribonucleic acid
FSH = follicle stimulating hormone
HCG = human chorionic gonadotropin
HMG = human menopausal gonadotropin
LH = luteinizing hormone
PMS = pregnant mare serum
PEP = phosphoenolpyruvate
PKU = phenylketonuria
PVP = polyvinylpyrrolidone

ized oocyte, *in vitro* fertilization and culture of the early embryo. It must be kept in mind, however, that these divisions are arbitrary and artificial; in reality, all of development is a continuum.

Oocyte Culture

Normal Oocyte Maturation and Its Relation to Ovulation

In female mammals, endogenous gonadotropins are secreted in a cyclic pattern, and repetitively bring a number of oocytes to the preovulatory condition. Basically, this involves an increase in the size of the oocytes, proliferation of the surrounding follicle cells and approximation of the follicular surface to the ovarian capsule. Then, at about the time of the midcycle surge of luteinizing hormone, two events take place: oocyte maturation and ovulation.[3]

To understand maturation, it must be recalled that in all known mammalian species, oogonia are transformed into oocytes only during a female's own fetal life or in the immediate newborn period; no new oocytes appear at any later time.[4,5] Each oocyte attains a follicle-cell investment, replicates its deoxyribonucleic acid (DNA) and enters prophase of the first meiotic division.[6] At this stage, the oocytes arrest, with their chromosomes drawn out long and threadlike.[7] They remain in this condition until they have undergone their preovulatory growth; then, just before ovulation, meiosis resumes. The chromosomes condense, complete the first meiotic division (with formation of the first polar body), and proceed as far as metaphase of the second meiotic division. Here, a second physiological arrest occurs. In most species, this is the point at which fertilization takes place, only after which does the second meiotic division proceed to completion. *Maturation* is the term given to the portion of meiosis between the two normal, physiological arrest points. It can be triggered not only by endogenous gonadotropins but also by exogenous hormones—usually injections of pregnant mare serum (PMS) and human chorionic gonadotropin (HCG).[3]

In most species, maturation occurs just before ovulation. The majority of mouse eggs are released from the ovary at second meiotic metaphase, although some of the earlier eggs to be ovulated are at earlier stages in meiosis, and complete maturation in the fallopian tube.[3] The maturation-ovulation relationship is quite constant for any given species. In mice, maturation begins two hours after the administration of HCG, and is completed by 14 hours, the time at which ovulation occurs. In humans, the onset of maturation takes place about 25 hours after HCG; by 36 to 43 hours, when ovulation occurs, maturation is complete. Depending upon the species, the interval between the onset of maturation and ovulation ranges from 10 to 24 hours.[3]

There are few mammalian exceptions to this maturation-ovulation relational sequence. In dogs, foxes and perhaps horses, ovulation precedes maturation; the latter presumably takes place in the tube.[3] The Madagascan insectivore Ericulus is even more unusual: after intraovarian maturation, sperm enter the ovary and fertilize the egg, only after which is the new embryo ovulated.[3]

A small number of matured, nonovulated oocytes can be found in the ovaries of most mammals.[3] This may represent the first step in oocyte atresia. In addition, maturation without ovulation may result from administration or release of smaller-than-usual quantities of HCG or luteinizing hormone (LH)—which would imply that less gonadotropin is necessary to initiate maturation than to cause ovulation.[8]

In Vitro *Oocyte Maturation in Nonhuman Species*

In vitro culture of unfertilized oocytes is possible because physical removal of an ovum from its follicle will also result in the initiation of maturation. In 1935 Pincus and Enzmann obtained rabbit oocytes from large ovarian follicles, and cultured the ova on plasma clots. Over the several hours of culture, many of the ova were observed to have extruded first polar bodies.[8] Two years

later Moricard and De Fonbrune similarly cultured mouse oocytes.[9]

Therefore, it became apparent that ovarian oocytes from large follicles could be carried through maturation if they were maintained in appropriate culture media under controlled environmental conditions. However, very little progress was made for a quarter of a century after Pincus and Enzmann's report. For one thing, there was great difficulty in obtaining satisfactory chromosome preparations for study from the matured eggs; this problem was not resolved until the late 1950's, with the tremendous breakthroughs in the technology of chromosomal preparation. The other drawback was the vagarious nature of the natural substances being used as culture media. Since the composition and toxicity of different batches of plasma and serum varied tremendously, consequently so did the rates of maturation.

A major step forward was the demonstration by W. K. Whitten in 1956 that eight-cell mouse embryos would undergo apparently normal development in a defined Krebs-Ringer balanced salt solution containing bicarbonate as a buffer, protein and a carbohydrate energy source.[10] By 1963 R. L. Brinster had redefined the medium so that it would support the development of two-cell mouse embryos.[11]

It was not a great jump from the culture of early embryos to that of prefertilization oocytes. The major early worker in this area was the English biologist R. G. Edwards. Between 1960 and 1965 Edwards published studies of the *in vitro* maturation of oocytes of several species, including rats, hamsters, pigs, cows, sheep, mice, monkeys and humans.[12-14] He tried several media, but used primarily the complex Medium 199, supplemented with 15 percent bovine serum, and antibiotics. During his experiments, Edwards was able to determine that the time sequence of *in vitro* maturation, as it is *in vivo,* is relatively constant and specific for any given species, but that there is considerable variation among species.[13]

By the mid-1960's, microbiochemical techniques had evolved to the point where J. D. Biggers, D. G. Whittingham and R. P. Donahue were able to study and report on the pattern of energy metabolism in the mouse oocyte.[15] Using the simple basic salt solution developed by Brinster, they were able to determine that for the oocyte, the optimal energy source was pyruvate; in fact, the only other compound tested that would serve at all satisfactorily in this capacity was oxaloacetate. Consequently (as we shall see) the oocyte is even more fastidious in its requirements than the two-cell embryo, which itself is more exacting than the eight-cell embryo. The authors concluded that the pattern of energy metabolism is determined in the oocyte before fertilization, and is very specific.

Biggers and his group also noticed that when the oocyte culture system included follicle cells, the energy-source requirements were not quite as specific; for example, glucose and phosphoenolpyruvate could then also support maturation.[15] This suggested that follicle cells may normally help to supply critical energy sources to the oocyte during the maturational period. This concept was followed up by Donahue, in work done with S. Stern. These investigators reported that in a culture system including follicle cells and using glucose as the sole energy source, not only would mouse oocytes mature but the follicle cells could indeed be shown to be producing pyruvate in amounts sufficient to support oocyte maturation.[16] Therefore the scientists concluded that *in vivo* either the stimulus to ovulation permitted follicle cells to initiate production of pyruvate so that maturation could begin, or it modified the oocyte in some fashion so as to permit it to react to the pyruvate being produced all along by the follicle cells.

Building upon the work of Brinster and of Biggers and associates, Donahue was able to carry out an hour-by-hour analysis of the maturation of the mouse oocyte *in vitro.*[17] In doing so, he correlated the progressive stages of meiotic chromosomal development with the culture interval after liberation of the oocyte from the follicle. By 11 hours, a significant number of ova had reached second meiotic metaphase; the peak number of 88 percent of all cultured oocytes did not reach this stage, however, until 17 hours of culture. Of the remaining 12 percent of oocytes, about half failed to initiate maturation, and half progressed only to first meiotic metaphase. No specific explanation has been offered to account for the failure of some cultured oocytes to complete maturation.

In succeeding years, the oocyte of the mouse has continued to be the favorite of developmental biologists, although attention has also been paid to the ova of rabbits,[18] guinea pigs,[19] domesticated farm mammals[20-22] and nonhuman primates.[23] Basically, the culture technique described by Brinster and adapted by Donahue has remained

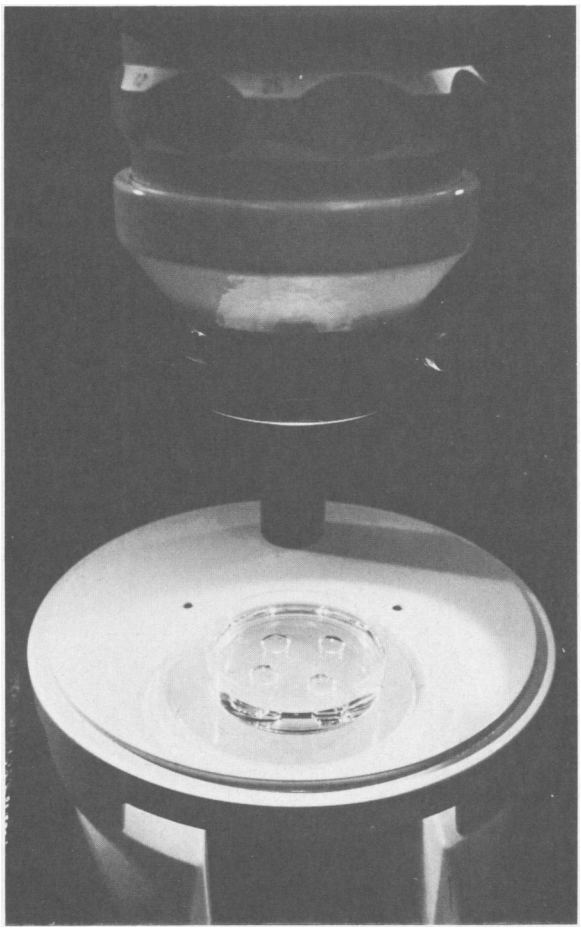

Figure 1.—Culture system for oocytes or embryos. Microdroplets of culture medium under paraffin oil, in plastic tissue-culture dish. The dish rests upon the stage of a dissecting microscope. (Photo by Jim Cummins.)

the standard.[17] Culture medium is maintained at a pH of approximately 7.2 by the use of a carbon dioxide/bicarbonate buffer system. Droplets of about 75 microliters' volume are placed at the bottom of plastic tissue-culture dishes, under a layer of sterilized paraffin or silicone oil (Figure 1). The major purpose of the oil is to act as a physical buffer, protecting against evaporation of the medium, or sudden changes in temperature or pH. The dishes are placed either in controlled gas-flow incubators or sealed desiccators, usually in an atmosphere of 5 percent carbon dioxide/95 percent air, and at a temperature of 37°C (close to normal body temperature for both mice and men). Many types of culture medium and several different atmospheric gas combinations have been utilized with this system. Although major modifications of the system have been tried,[24,25] the great majority of the work has been done utilizing the basic described technique. (The course of mouse oocyte maturation and fertilization *in vitro* is shown in Figure 2.)

Are oocytes matured *in vitro* normal and comparable to ova which have matured *in vivo?* Several measurements suggest that the two groups may be identical in many but perhaps not all respects.

For one thing, in all examined species the time interval spent in maturation appears to be about the same whether the ova mature *in vivo* or *in vitro.*[13,17] For example, Donahue found that natural maturation is complete in mice by the time of ovulation, 14 hours after the administration of HCG. This stands in comparison with the maximum of 17 hours needed for *in vitro* maturation in this species. In addition, ultrastructural studies of mouse oocytes matured either *in vivo* or *in vitro* showed no significant differences between the two groups.[26,27]

The ultimate test of normality of maturation probably is the degree to which eggs can be fertilized and subsequently develop into normal young. Oocytes matured *in vitro* generally have not been as capable of being fertilized as those matured *in vivo.* In mice, Cross and Brinster showed that the rate of fertilization *in vitro* was twice as high when ova were used that had been matured *in vivo* rather than *in vitro.*[28] It is uncertain whether the lessened capacity for fertilization shown by oocytes matured *in vitro* would be associated with offspring having greater numbers of birth defects, but there is no evidence to suggest that this would be so. Of particular interest in this respect would be a controlled study of the incidence of chromosomal abnormalities in oocytes matured *in vivo* and *in vitro.*

In Vitro *Oocyte Maturation in Humans*

The first report of human oocyte maturation *in vitro* is that of Pincus and Saunders in 1939.[29] Using human serum as the culture medium, these workers noted formation of polar bodies in 19 percent of their experimental ova, after culture periods of up to 72 hours.

There were no further reports of successful attempts at culturing human oocytes until 1965, when R. G. Edwards reported recovery from large ovarian follicles of 250 oocytes, 133 of which were examined after varying intervals of culture in Medium 199 with 15 percent fetal calf

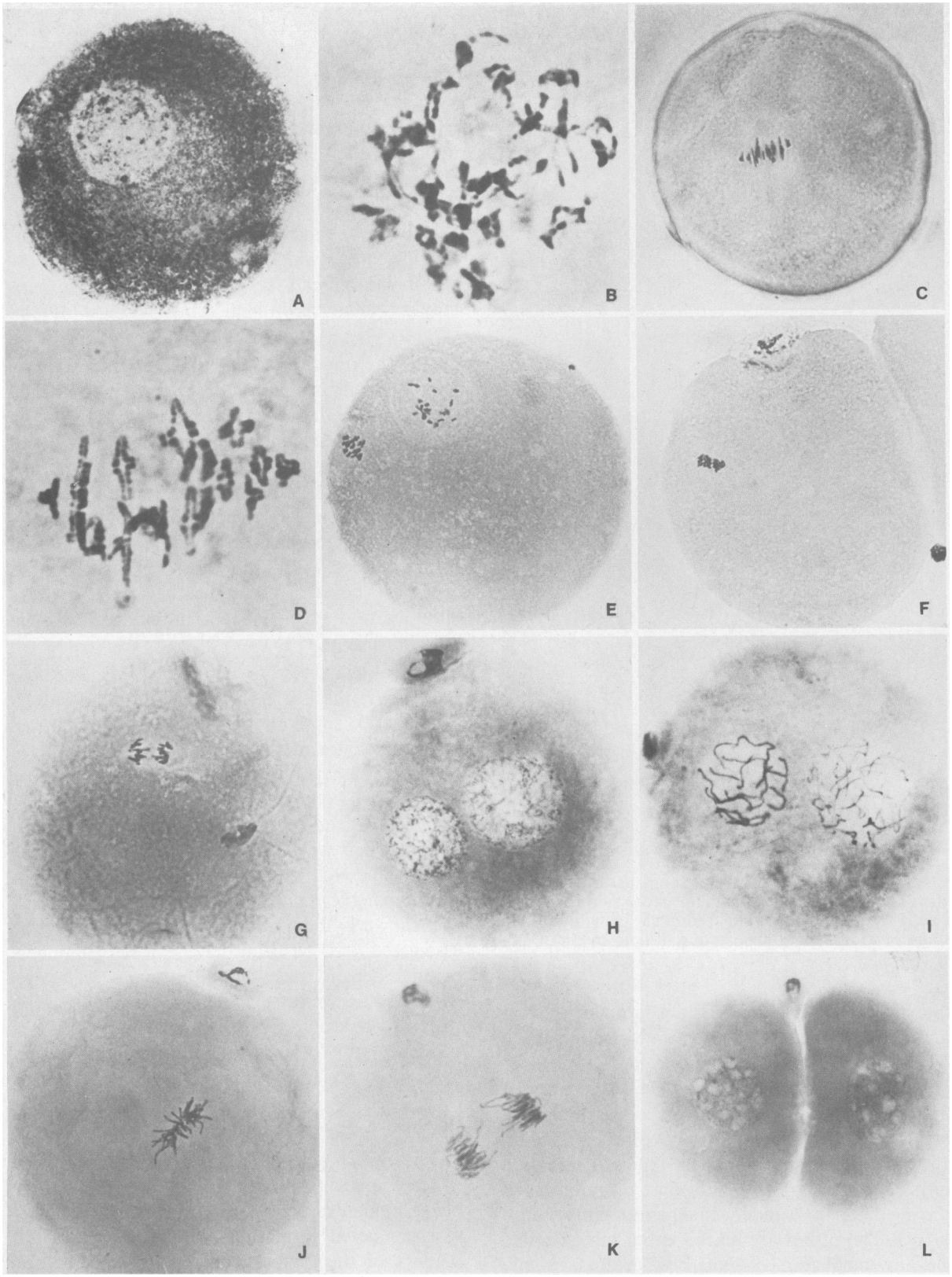

Figure 2.—The course of mouse oocyte maturation and fertilization *in vitro*. (Zona pellucida dissolved by fixative.) (Reproduced by permission from Journal of Experimental Zoology: Figures A-F, 169:237-250, 1968; Figures G-L, 180:305-318, 1972; courtesy Wistar Institute Press.)

A, mouse oocyte after liberation from follicle. Nucleus (germinal vesicle) in prophase; individual chromosomes cannot be visualized.

B, formation of chromosomes within nucleus of oocyte, before meiotic spindle formation. Two hours of culture.

C, first meiotic metaphase. Nine hours of culture.

D, high-power view of chromosomes at first meiotic metaphase.

E, first meiotic division completed. Note typical disorganization of chromosomes in polar body. Approximately 11 hours of culture.

F, second meiotic metaphase. Oocyte chromosomes organized on spindle; polar body and its chromosomes (seen at 12 o'clock) already show degeneration. 17 hours of culture. Arrest at this point pending fertilization.

G, sperm head has entered oocyte, and is beginning to swell and transform into male pronucleus. Maternal chromosomes have been activated, and are in the process of completing the second meiotic division.

H, fertilized oocyte showing (large) male and (small) female pronuclei. Second polar body is seen, with degenerating chromatin. (First polar body has since degenerated.)

I, chromosomes beginning to condense and appear in pronuclei, preparatory to first mitotic (somatic, cleavage) metaphase.

J, first mitotic metaphase, representing first actual physical contact of maternal and paternal hereditary material.

K, separation at anaphase of chromosomes, as one-cell embryo divides into two cells.

L, two-cell mouse embryo. Second polar body visible at 12 o'clock.

serum.[14] In this study Edwards was able to outline the temporal sequence of maturation for human oocytes, much as Donahue was later to do (with greater precision) for mice. He found that maturation was initiated approximately 25 hours after the onset of culture, that first meiotic metaphase occurred between 28 and 35 hours, and that second metaphase was reached between 36 and 43 hours. He commented on the prevalence of abnormal arrest at the onset of maturation and at first metaphase (though he referred to the latter as "anaphase block").

Although Jagiello, Karnicki and Ryan cultured 73 percent of 30 human oocytes to second metaphase, the ova were obtained at surgical operation, 24 hours after HCG had been given.[30] Therefore the impetus to initiate maturation had come *in vivo.*

The next report of complete *in vitro* maturation of human oocytes was that of J. F. Kennedy and R. P. Donahue, in 1969.[31] These investigators obtained 426 oocytes from surgically-excised ovaries, and attempted to culture them in media of differing complexities. They found that the rate of maturation was highest in F10 medium, intermediate in Medium 199, and lowest in the simple Krebs-Ringer salt solution with pyruvate which works so well with mouse oocytes. Altogether, only 45 percent of the oocytes reached second metaphase. It was also noted that removal of the follicle cells (cumulus) which normally adhere to the oocytes resulted in lower rates of maturation, but it could not be determined whether this was due to damage to the oocytes caused by follicle-cell removal, provision of a necessary nutrient by the follicle cells or a protective (detoxification) effect of the follicle cells.

Since 1969 other attempts have been made to culture human oocytes *in vitro,* but with no greater success than noted in earlier reports.[32,33] Human ova may be more fastidious in their culture requirements; alternatively, there may be fewer follicles of sufficient size at any one time in human ovaries than in mouse ovaries. In any case, even when human oocytes are successfully matured *in vitro,* they are, like mouse oocytes, more difficult to fertilize *in vitro* than *in vivo.*[34,35] This fact notwithstanding, results of ultrastructural studies have failed to show any morphological differences between human oocytes matured *in vivo* and *in vitro.*[36] Moreover the temporal sequence of maturation of human ova appears to be similar *in vivo* and *in vitro.*[14,37]

In Vitro Fertilization

Aspects of Natural Fertilization

Fertilization is a more complex sequence of events than is generally realized. Normally it begins with penetration of the egg by one, and only one, competent spermatozoon. This is followed by completion of meiosis in the ovum, with reduction of the chromosome number to the haploid condition; replication of DNA by both the male and the female haploid groups of chromosomes, and finally syngamy—or the union of the maternal and paternal chromosomes. This is rapidly followed by the first somatic, or cleavage, or mitotic division, which results in the formation of a two-cell embryo. (See Figure 2 for course of mouse maturation and fertilization *in vitro.*)

Most of the studies concerning the nature of the fertilization process have been done on the gametes of rabbits and laboratory rodents: the process appears to be quite similar in all species. Findings in electronmicroscopic studies[38,39] have shown that numerous spermatozoa make their way through the investing cumulus cells, presumably by release of enzymes from the region of the acrosome ("head-cap"), which is thought to be a modified lysosome. Two acrosomal enzymes have been identified: hyaluronidase and a trypsin-like acrosomal proteinase. In most species, only one sperm usually manages to penetrate the mucoprotein zona pellucida, which surrounds the oocyte proper. This zonal penetration is probably also achieved through the mediation of enzymatic digestion, the sperm following a curved course through the zona, and leaving a *penetration slit.* Upon reaching the oocyte surface, the portion of the sperm membrane at the posterior aspect of the head fuses with the ovular (vitelline) membrane, and then the rostral part of the sperm head in drawn into the egg by progressive membrane fusion and phagocytosis.

Next, a nuclear envelope surrounds the chromatin in the sperm head, thus forming the *male pronucleus.* The rest of the spermatozoon eventually disintegrates. Simultaneously, the oocyte extrudes the second polar body, completes the second meiotic division, and the chromatin becomes the *female pronucleus.* In all mammals, the two pronuclei remain separate, merely approaching each other as they replicate their chromatin.[40] Thus, there is no actual fusion of maternal and paternal pronuclei until the pronuclear membranes break down, a spindle forms, and the two

groups of chromosomes come together at metaphase of the first mitotic division. Division follows rapidly, with formation of a two-cell embryo.

It appears that before normal fertilization can occur in mammalian species, certain specific preparatory changes must take place in both the egg and the sperm. Around the time of ovulation, the ovum accumulates a complement of cortical granules: small, membrane-surrounded packages of mucopolysaccharides and hydrolases.[39] These cortical granules originate in the Golgi complexes and then move peripherally to lie directly beneath the vitelline membrane. Upon entry of the fertilizing spermatozoon, the granules are extruded from the egg, releasing their contents.[41] This is referred to as the *cortical reaction.* It is believed that the enzymes from the cortical granules modify the oocyte investments in such a way that they become impervious to further sperm penetration, thereby providing a safeguard against the development of embryos with abnormal numbers of chromosomes.[39]

The prefertilization changes in the sperm are generically referred to as *capacitation.* In 1951 Chang and Austin independently noted that freshly-ejaculated or epididymal sperm were not capable of fertilizing oocytes, but that sperm which had been preincubated for a few hours in the female reproductive tract acquired fertilization competence.[42,43] Even today, the precise nature of capacitation is obscure; however, the phenomenon may be related to removal or alteration of seminal plasma components which are bound on the surface of the sperm head.[44]

The necessity for capacitation seems to have been well established in many animal species, including rats, mice, rabbits, hamsters, sheep, ferrets and cats.[44] In primates, the evidence for its necessity is less definite.[44] In addition, incubation in the female genital tract does not appear to be specifically required for successful capacitation: in different species, capacitation has been achieved *in vitro* by incubation in follicular, tubal or uterine fluid; in serum; in solutions of β-glucuronidase, and in simple chemically-defined media, and even by exposure to Sendai virus.[44]

The morphological correlate of capacitation is the so-called acrosomal reaction. First reported in 1958 by Austin and Bishop,[45] and further elaborated in 1963 by Austin,[46] this consists of dissolution of the outer membrane of the acrosome, with subsequent loss of this structure down to the inner acrosomal membrane. Therefore it can be seen that the acrosomal reaction would result in exposure of the immediate environment to hyaluronidase and acrosomal proteinase. These enzymes are thought to play a role in the dissolution of the intercellular matrix of the cumulus and the local digestion of the zona pellucida.[47] It is of interest that in rats and mice, capacitation is not necessary for fertilization of eggs which have been stripped of their cumulus cells and zonae; fresh epididymal sperm have no trouble in penetrating these naked oocytes.[48,49]

In Vitro *Fertilization of Nonhuman Species*

In 1959 Chang described *in vitro* fertilization of rabbit eggs, which was followed by the birth of normal young.[50] Since then, as summarized by Toyoda and Chang, the oocytes of mice, guinea pigs, rats, cats, pigs and humans have also been fertilized *in vitro.*[51]

The basic technology used for *in vitro* fertilization has been quite similar from report to report, and in fact does not differ significantly from the procedure employed for *in vitro* oocyte maturation. Culture medium is set up in microdroplets under oil in tissue culture dishes, or is layered under oil in a watch glass. The sperm and ova are placed into the medium, which is then incubated for between 4 and 24 hours, usually at 37°C and in an atmosphere of either 5 percent carbon dioxide/95 percent air or 5 percent carbon dioxide/5 percent oxygen/90 percent nitrogen. Most reports have mentioned that the optimal pH is somewhat higher than that for oocyte maturation, about 7.6 to 7.8.[52] However, in mice, Iwamatsu and Chang found that pH 6 to 7 gave the best results.[53]

Criteria for successful fertilization *in vitro* have become stringent with the passage of time. The only absolute measures of success, of course, are the demonstration of a Y chromosome in the embryo, or birth of male offspring after transfer to a recipient uterus. However, the following morphologic parameters are frequently accepted as constituting evidence of fertilization: visualization of the second polar body, a sperm head in the process of transforming into a male pronucleus, and a degenerating sperm mid-piece and tail in the cytoplasm; or observation of the polar body and the fully-formed characteristic male and female pronuclei. (In some species, the male pronucleus is larger than the female.[54]) Although some workers have also accepted apparently-normal cleavage divisions as proof of fertilization,

others disagree because of the possibility that this may represent either degenerative fragmentation or the initial stages of parthenogenetic development—a process known to occur after many types of experimental manipulations of oocytes.[55,56]

A recent ultrastructural study showed that fertilization of mouse eggs *in vitro* produces embryos highly comparable to those resulting from natural fertilization.[57] The culture medium was a Krebs-Ringer bicarbonate solution, modified from Whittingham's formula. At all stages during and after fertilization, the zygotes had very similar morphology. Only minor differences could be seen, and these could not be called abnormal. The actual event of sperm penetration appeared identical *in vivo* and *in vitro*. Furthermore, the timing of the pronuclear changes, syngamy and the first mitotic division was identical to that described by Donahue after natural fertilization.[54]

On the other hand, rates of fertilization are lower *in vitro* than *in vivo*. Although higher rates have been reported,[58] usually, no more than 50 percent of oocytes become fertilized *in vitro*. This may reflect either suboptimal selection of oocytes or the fact that the ideal conditions for *in vitro* fertilization have yet to be discovered. It does not necessarily imply abnormality in the fertilized ova.

Another point of difference between fertilization *in vivo* and *in vitro* was discovered by Niwa and Chang, working with rat gametes.[59] In contrast to earlier work by Blaudau and Odor and by Austin, which showed that at the time of *in vivo* fertilization there are approximately 10 sperm in the tube for each ovulated egg, Niwa and Chang needed 3,000 to 6,000 sperm per egg to effect optimal fertilization *in vitro*. No clear reason could be put forth for this large concentration difference, which probably holds generally true for other species as well; different workers studying different species have determined the most satisfactory concentration of spermatozoa for *in vitro* fertilization to be between about 10^5 and 10^6 cells per ml of culture medium.

Although detailed investigations have not been made of the incidence of congenital anomalies in offspring after *in vitro* fertilization and transfer of embryos to foster mothers, most reports have not mentioned any increase over the rates seen after fertilization *in vivo*. An exception was the paper by Toyoda and Chang.[51] These workers fertilized rat ova in a simple defined medium and obtained 88 percent success. In the embryos which were then transferred and developed to term, there was a 50 percent incidence of microphthalmia, sometimes involving only one eye per animal. No other anomalies were seen and the peculiar cluster could not be explained.

In Vitro *Fertilization of Human Oocytes*

The first attempt to fertilize human ova *in vitro* was made by Menkin and Rock.[60] They recovered 800 eggs from ovarian follicles and tried to mature them in cultures of human serum. After approximately 24 hours, 138 of these oocytes were exposed to freshly-ejaculated human sperm in a simple salt solution, over a one- to two-hour period. Then the ova were placed back in serum. Three ova were considered by the authors to have undergone apparently normal cleavage over the 40 to 46 hours following fertilization. However, degenerative fragmentation and rudimentary parthenogenesis were not ruled out.

In 1966 Edwards and his associates tried to fertilize human oocytes that had been matured in Medium 199 with 15 percent inactivated fetal calf serum.[34] They attempted to capacitate the sperm by both *in vivo* and *in vitro* modalities: placing the sperm in rabbit uteri and tubes, recovering sperm in cervical mucus after intercourse or culturing sperm samples with small pieces of human fallopian tube. In addition the sperm were washed twice to remove seminal plasma. After 36 to 40 hours of maturational culture, the eggs were exposed to the sperm for 8½ to 42 hours, and then examined. Fertilization could not be confirmed, however, in any of the 104 exposed oocytes. In addition, 20 more oocytes were inseminated in the reproductive tracts of rabbits, and 67 in monkeys. Very few of these eggs could be recovered and none were fertilized.

By 1969 Edwards was working with a new group of investigators.[35] They used a modified Tyrode solution for capacitation, and matured the oocytes in follicular fluid alone or in combination with Hanks', Brinster's or Bavister's medium. Fertilization was carried out in Bavister's medium, and success was inferred by the finding of pronuclei and sperm midpieces, and extrusion of second polar bodies. Despite meticulous monitoring of culture components, pH and other environmental factors, only 34 of 56 cultured oocytes matured. Then, after insemination, only seven were found to have pronuclei. Even worse, some of the pronuclei were abnormal. Furthermore, five percent of control oocytes underwent

cleavage-like parthenogenetic development. The authors voiced concern regarding the poor maturation rate and possible abnormality of eggs matured totally *in vitro,* and changed their experimental course to the attempted fertilization *in vitro* of oocytes matured *in vivo.*

As a means of obtaining matured oocytes for fertilization, Edwards joined forces with Patrick Steptoe, a gynecologist skilled in laparoscopy.[37] To time ovulation, women were given injections of human menopausal gonadotropin (HMG) and HCG; the optimal schedule was determined to be three injections of 225 IU HMG between days 2 and 9 of the menstrual cycle, and a single dose of 5,000 IU HCG between the 9th and 11th day. A group of 46 infertility patients served as subjects. Steptoe carried out laparoscopy between 29 and 31 hours after HCG administration, based upon Edwards' previously-cited data on oocyte maturation. Using an aspiration device of original design, Steptoe eventually was able to recover approximately one preovulatory oocyte per ovary. Best results came from follicles with diameters between 1 and 2 cm.

The oocytes recovered by Steptoe, which were mostly at or near first meiotic metaphase, were incubated for one to four hours in a mixture of follicular fluid and the fertilization medium being tested: Whittingham's or modifications of Waymouth's, Ham's F10 or Bavister's media, all at pH 7.5 to 7.6.[61] Next, both sperm and eggs were washed with fresh fertilization medium, and then they were incubated together for 12 to 15 hours. At this point, the embryos were transferred to medium which had been designed to support cleavage: Whittingham's, Whitten's, Waymouth's, 199 or Ham's F10—all modified to some degree and all adjusted to pH 7.3. Criteria for fertilization included the observation of two pronuclei, two polar bodies and a sperm tail in stained preparations; two pronuclei in the live egg, or morphologically-normal cleavage of the zygote, preferably after having observed pronuclei.

The number of embryos transferred from the fertilization to the cleavage medium was not mentioned, but 38 embryos were reported to have cleaved in culture up to the 16-cell stage, and one or more embryos were produced from 29 of 49 patients undergoing laparoscopy. Fertilization was achieved in all media tried except Ham's F10; however, this medium, with 20 percent inactivated fetal calf serum, seemed optimal for support of the cleavage embryos, when the osmolality was raised from the conventional 287 mOsm per kg to 300 to 305 mOsm per kg. Based on his observations, Edwards offered the hypothesis that the follicle cells of the cumulus might themselves effect sperm capacitation through the production of progesterone, a hormone capable of destabilizing lysosomal membranes such as are found in the acrosome.

Luigi Mastroianni's group has also been active in this area. Seitz and co-workers cultured 16 human ova in F10 medium, and inseminated them with human sperm that had been washed and then incubated in a monkey uterus.[62] Six ova were considered to have been fertilized and to have cleaved to stages from two to eight cells.

In 1973, Soupart and Morgenstern reported on the attempted maturation and fertilization *in vitro* of 44 human oocytes.[63] The maturation culture was composed of one part Bavister's medium and three parts follicular fluid; the fertilization medium was Bavister's adjusted to pH 7.6. The addition of follicle-stimulating hormone (FSH), LH and HCG to the fertilization medium appeared to increase the incidence of sperm penetration into and through the zona pellucida. This was interpreted as a capacitation effect, apparently supporting Edwards' hypothesis, in that the action of the gonadotropins might have been expressed via mediation of follicular-cell hormone production. Overall, however, only one oocyte appeared to have been fertilized.

After having used Edwards' and Steptoe's techniques for *in vitro* fertilization, an Australian team of physicians reported having transferred a human zygote at the 6- to 8-cell stage. However, menstruation occurred nine days after transfer.[64]

It is still impossible to comment on the normality of human oocytes fertilized *in vitro.* To this point, the applied criteria for fertilization have all been only presumptive, and very few oocytes have fulfilled them. No Y chromosomes have been identified in early human cleavage embryos, and although fertilization most likely did occur when ova were found to have two pronuclei, two polar bodies and a degenerating sperm mid-piece and/or tail, this still does not absolutely rule out parthenogenesis—especially in view of Edwards' finding of five percent spontaneous parthenogenetic development of control oocytes,[34] and the fact that parthenogenesis can be induced in the mouse by hyaluronidase. Furthermore, no embryos have progressed far enough (that is, beyond implantation) to observe anomalies of bio-

chemical or structural development. And in the absence of karyotypes of these embryos, we cannot rule on their chromosomal normality or abnormality. Soupart and Strong reported on the comparative ultrastructural features of a human tubal pronuclear egg, and oocytes fertilized *in vitro*.[65] They found no basic ultrastructural differences, but did notice some vacuolization of the cultured zygotes, suggestive of degeneration and therefore also suggesting that at least their own technique of ovum culture was not yet ideal.

Embryo Culture

Postfertilization Culture of Nonhuman Embryos

As reviewed by Biggers and Stern,[66] the first recorded attempt to culture mammalian embryos was that of Brachet, who in 1912 and 1913 maintained five-day rabbit embryos on plasma clots. Lewis and Gregory in 1929 also tried to culture rabbit embryos in plasma. In 1930 Gregory Pincus succeeded in supporting cleavage of rabbit embryos in several culture media, and in 1934 Pincus and E. V. Enzmann carried out the first transfer of cultured embryos into the uteri of recipient does, obtaining live, healthy offspring.

However, as we mentioned during the discussion of oocyte maturation, it was primarily the work of W. K. Whitten, R. L. Brinster and J. D. Biggers and his group that really opened up the field of embryo culture in particular, and preimplantational ectogenesis in general. Whitten and Brinster showed that cleavage could be obtained in simple defined media. Then, these investigators and others proceeded to use this information to investigate the metabolic requirements of mammalian embryos at different stages of cleavage. The acquisition of such knowledge led in turn to the development of more satisfactory and reproducible methods of culture.[67]

By far the greatest amount of work in postfertilization embryo culture has been done on mice, and rabbits are all alone in second place. Interest has also been shown in other small laboratory mammals and, because of the potential applicability to practical animal husbandry, in sheep and cows.

Brinster's 1963 paper is still the classic in the field.[11] In it, Brinster described the technically-simple method of embryo culture in microdroplets of medium under paraffin oil in a tissue-culture dish. The medium was a simple Krebs-Ringer salt solution, with bicarbonate as a buffer, bovine serum albumin as a protein source and stabilizing agent, sodium lactate as the energy source and antibiotics to guard against contamination. By the use of this system, Brinster was able to culture two-cell mouse embryos, obtained from the fallopian tubes, all the way to preimplantation blastocysts. Furthermore, he determined that the rate of development was highly reproducible and closely paralleled that *in vivo*.

Brinster followed up his initial work with several publications in which he manipulated his culture conditions in order to determine various optima, as evaluated by the number of two-cell embryos which would cleave to blastocysts. The best osmolarity was found to be 276 mOsm per kg, and the most satisfactory pH 6.82—this in spite of the facts that rat and rabbit tubal fluids are alkaline and that all of Brinster's work where pH was not a variable was carried out at pH 7.38.[68]

Brinster's experiments indicated that a fixed-nitrogen source was necessary for cleavage; bovine serum albumin at a concentration of 1 mg per ml seem optimal.[69] The albumin could also be replaced by equivalent quantities of the constituent amino acids. When this was done, however, a nonprotein polymer such as polyvinylpyrrolidone (PVP), acacia, dextran or Ficoll had to be added; otherwise, the embryos tended to float to the surface of the droplets. Omission of single amino acids was well tolerated, with the sole exception of cystine. In opposition to Brinster's data, however, Cholewa and Whitten were able to obtain cleavage of two-cell mouse embryos in the absence of a fixed-nitrogen source.[70]

Studies of energy requirements of the cleaving mouse embryo have showed a general trend toward less restrictive needs with increasing development. As previously mentioned, the unfertilized oocyte will undergo maturation only when supplied with either pyruvate or oxaloacetate. This great selectivity holds true for the first cleavage division as well, only pyruvate or oxaloacetate being able to serve as energy sources for the pronuclear zygote.[15] (However, lactate or phosphoenolpyruvate [PEP] will prevent degeneration of embryos for up to 48 hours of culture, and if pyruvate is added after 24 hours of culture in lactate, the embryos will then complete the first cleavage division.[71]) By the time the ovum has reached the late two-cell stage, lactate and phosphoenolpyruvate will function fully as sources of energy to support cleavage of the 2-cell embryos to the blastocyst stage.[72] In fact, a combination of

pyruvate and lactate may give the best rates of cleavage.[73] By the eight-cell stage, as had originally been reported by Whitten, glucose can support further development; in fact, cleavage will take place in the presence of *either* glucose *or* albumin.[74] This progressive change in energy requirements suggests corresponding developmental changes in the embryo during the preimplantational period. There may occur alterations in cell permeability to different compounds. In addition, changes occur in the activities of several enzymes, as summarized by Biggers and Stern.[66]

Even oxygen concentration in the culture atmosphere has come under scrutiny. Auerbach and Brinster determined that two-cell mouse embryos will proceed normally to blastocysts as long as the oxygen concentration is at least 1 percent.[75]

Again, the issue of normality arises. With regard to cultured mouse embryos, this question has been examined primarily on a functional basis.

Bowman and McLaren determined that *in vivo,* mouse embryos between the two-cell and the blastocyst stages had a cell doubling time of ten hours. In the early stages of culture, this rate was identical, but subsequently fell off to about 24 hours.[76] In addition it was determined that only 20 to 30 percent of cultured mouse embryos resulted in live fetuses at 17 days after transfer of blastocysts, compared with 50 percent success when the blastocysts were taken from the uterus.[77] There was an excess of both preimplantational and postimplantational mortality, and the surviving fetuses from the cultured embryos were about 100 mg lighter than the controls. Furthermore, there seemed to be an inverse relationship between the duration of culture and the viability and weight of the fetuses. Menke and McLaren also discovered that blastocysts maintained *in vitro* had a lower metabolic rate than those taken from the uterus.[78]

The first report of total *in vitro* preimplantational development leading to the birth of live young was that of A. B. Mukherjee in 1972.[79] Oocyte maturation, sperm capacitation, fertilization and cleavage to the blastocyst stage were all carried out *in vitro.* Previously, the nearest anyone had come to achieving successful total preimplantational ectogenesis was described by the report of Cross and Brinster, who matured mouse oocytes *in vitro,* and fertilized them *in vitro* with sperm capacitated *in vivo.*[28] After transfer, live fetuses were found upon autopsy of the recipients at 15 days' gestation.

Mukherjee matured mouse oocytes in Ham's F10 or in modified Whitten-Biggers Medium. Sperm were obtained from the epididymis, and capacitated by four hours' incubation in a 1:2 mixture of heat-inactivated follicular fluid and human tubal fluid. Fertilization was carried out by exposure of oocytes for 24 hours to capacitated sperm cells in Ham's F10 medium under paraffin oil. The embryos were then cultured for three days in fresh medium. At this point the blastocysts were transferred into the uteri of mice made pseudopregnant by mating three days previously with vasectomized males. Transfer was achieved by puncturing the utero-tubal junction with a micropipet, and blowing in the embryos. To be certain that any liveborn young had indeed originated from the ectogenetic embryos, the sperm and oocytes were taken from a strain of mouse with a coat color different from that of the recipient females and the vasectomized males to which they had been mated.

Not only is Mukherjee's report the first of its kind, it is also to date the only one. Furthermore, consideration should be given to the low rate of success he obtained. Of 325 oocytes placed into maturation culture, 195 showed formation of the first polar body, 140 were placed into fertilization medium, 32 two-cell embryos resulted and 11 blastocysts were recovered for transfer. Eventually, five liveborn, apparently normal, progeny were obtained—an ultimate yield of only about 1½ percent of the ovarian oocytes initially placed into culture.

Rabbit embryos have received less attention than those of mice. Ogawa and co-workers, using a highly complex medium, were able to culture 58 of 67 one-cell embryos to the eight-cell, morula or blastocyst stages.[80] Kane obtained an 81 percent yield of blastocysts from one-cell embryos cultured in a medium devoid of any carbohydrate energy source; it could not be discerned whether the embryos were using endogenous energy sources or the amino acids present in the medium.[81] Brackett and associates used ova and sperm matured and capacitated *in vivo,* carried out *in vitro* fertilization and obtained apparently normal cleavage in 43.7 percent of 302 oocytes.[82] In all, 22.6 percent of 31 transferred cleaved ova developed to term birth in genetically-dissimilar recipients.

Van Blerkom and co-workers employed ultrastructural and microbiochemical techniques to compare preimplantational rabbit embryos which

had cleaved *in vivo* and *in vitro*. No differences were seen using an electron microscope during the first five days of embryogenesis.[83] Then, a combination of electrophoresis and radioautography showed that qualitative patterns of protein synthesis *in vitro* paralleled those *in vivo,* stage by stage.[84]

Tervit and co-workers recovered sheep and cattle embryos from the tube at the one-cell to the eight-cell stages and cultured them in a medium based upon the composition of sheep oviductal fluid.[85] After three to six days in culture, some of the embryos were transferred to recipients. In the sheep, a small number of viable embryos were found at slaughter of the recipients on day 13 or 14 of pregnancy. One of the recipient cows was found to be pregnant, as diagnosed by rectal palpation on day 35. This animal went to term, but no mention was made of the offspring.

A concept which has received much popular attention is that of the freezing of embryos. In 1971, Whittingham recovered mouse embryos at either the eight-cell or the blastocyst stage, and froze them at −79°C for 30 minutes in phosphate-buffered saline with 15 percent polyvinylpyrrolidone, an agent which protected the embryos against rupture.[86] After thawing, 75 percent of the eight-cell embryos appeared normal and 52 percent developed into blastocysts after 48 hours of culture. After thawing and a subsequent 24 hours of culture, 62 percent of the frozen blastocysts were considered normal. Of 19 transferred eight-cell embryos, 21 percent developed into normal offspring, as did 69 percent of 13 transferred blastocysts.

A year later, Whittingham extended his work.[87] He froze more than 2,500 mouse embryos, from the one-cell to the blastocyst stage, for up to eight days, at either −196° or −269°C. Dimethylsulfoxide (DMSO) or glycerol was added as a protective agent, and freezing and thawing were both done at very slow rates. Depending upon the original stage when frozen, from 50 to 70 percent of the embryos developed into normal preimplantation blastocysts when thawed and cultured in Whittingham's medium. Approximately 1,000 of the survivors were transferred to virgin recipients, 65 percent of which became pregnant —a rate equal to that achieved after natural insemination of virgin mice. In the mice which did conceive, 43 percent of the transferred embryos developed into apparently normal term fetuses or live young.

In further experiments, Whittingham and Whitten were able to freeze embryos for up to eight months, transfer them by air from Bar Harbor, Maine, to Cambridge, Massachusetts, and obtain live births after transfer to recipients.[88]

Less work of this sort has been done with larger animals. Moore and Bilton recovered sheep embryos at the one- to four-cell stages, and as morulae.[89] These embryos were cooled to 5°C for ten minutes to six hours, after which they were cultured for 48 hours. Approximately 70 percent of the rewarmed embryos progressed normally in culture. Furthermore, of 30 cooled-and-warmed embryos that reached the blastocyst stage, and were then transferred to recipient ewes, 11 developed into liveborn lambs.

Human Embryo Culture

Some of the work done to date in human embryo culture has been described previously, in connection with *in vitro* fertilization. An additional report by Steptoe, Edwards and Purdy that is worthy of note appeared in *Nature* in 1971.[90] There, they described the extended preimplantational culture of six human embryos, utilizing already-described techniques of fertilization and cleavage culture. Two embryos developed only to the morula stage; they were found to have 23 and 30 cells. Two other embryos formed blastocele cavities, but the entire embryos appeared abnormal, and in fact were found to contain only 16 and 20 cells. The last two embryos developed into apparently normal blastocysts. One was examined at 159 hours of culture, and was found to have 110 nuclei. The other blastocyst was treated at 147 hours so that its cells might be karyotyped. It was found to have 112 cells, but no chromosomal spreads were suitable for karyotyping. The authors estimated that the cells probably were diploid.

The team of Seitz, Rocha, Brackett and Mastroianni matured 50 follicular oocytes for 24 hours at F10 medium, with 20 percent heated human female serum, and 25 micrograms of sodium estrone sulfate per ml.[62] Sperm were capacitated in the uterus of a midcycle rhesus monkey. After fertilization, the embryos were transferred to fresh medium identical to that used for maturation. Of the 50 original ova, 8 apparently underwent cleavage, reaching stages between 2 and 12 cells. No cleavage was seen among 20

control oocytes which had been incubated with either washed, ejaculated sperm or sperm-free washings from a monkey uterus. Therefore, it was concluded that capacitation is indeed necessary in humans. Cited by the authors as evidence of fertilization were: sperm in and around the zona pellucida, visualization of two polar bodies, observation of "chromatin" in some of the blastomeres, progressive cleavage stages and failure of the controls to cleave.

Implications of Preimplantational Ectogenesis

Both in scientific journals and the lay press, preimplantational ectogenesis has been the subject of considerable discussion and controversy. Some of this has been sober, but a good deal more has been ill-founded in scientific fact, emotional and even hysterical. Preimplantational ectogenesis deals intimately with basic questions of human life and human reproduction, which forces anyone who would consider the issues to think through his basic concepts of right and wrong, usually a very painful process.

Preimplantational Ectogenesis in Clinical Medicine

The most frequently-discussed application, and in fact the expressed goal of Edwards' and Steptoe's work, is the relief of infertility caused by obstruction of the fallopian tubes. A very large number of women suffer from this problem, and so would be candidates for laparoscopic recovery of oocytes, *in vitro* fertilization with the sperm of the husband and subsequent reimplantation into the wife's uterus.

Two of the leaders of the opposition to this procedure are theologian Paul Ramsey and biologist-ethicist Leon Kass. Referring to *in vitro* fertilization as "unethical experimentation on the unborn,"[2] Kass argues that on ethical grounds such work cannot be justified. First, he says, the risk cannot be determined without carrying out the procedure on humans, and second the prospective fetus is incapable of giving informed consent.

Part of the opposing argument to Kass' position has been presented by ethicist Marc Lappé.[91] Lappé argues that the small amount of animal data available do not suggest that the risk of *in vitro* fertilization is large. Nor might one really expect it to be, after manipulation of these embryos which have not yet reached the period of organogenesis. Lappé also reminds us that no official effort has been made to prohibit reproduction by two carriers of autosomal recessive diseases like phenylketonuria (PKU) or sickle cell anemia, where the 25 percent risk of affected offspring would almost certainly exceed any risk associated with *in vitro* fertilization. This argument could be extended to point out that 3 to 5 percent of all newborn infants resulting from *in vivo* fertilization suffer from some kind of birth defect and that children conceived by this technique do not give informed consent. If prospective parents can give consent for *in vivo* fertilization, it is difficult to understand why it could not be given for the *in vitro* procedure as well.

However, Lappé does point out that with the present dearth of reliable safety data, it might be a good idea to accumulate more information in animal experiments, before recommending that this work be done in humans. If findings from extensive animal experimentation—including primate work—are reassuring, then preimplantational ectogenesis might be offered, with appropriate explanation, to individual infertile couples. This manner of procedure would not be different from any other developing medical undertaking.

What of the possible psychological effects of human *in vitro* preimplantational development? It is quite certain that the first offspring to be produced in this manner will attain celebrity status. However, this does not appear to be a fatal disease. In addition, one might wonder whether parents would relate in a healthy fashion to such children. We know of no evidence to suggest the contrary.

The question may arise as to the disposal of "extra" ova and embryos. Suppose four eggs should be obtained from the ovaries of an infertile woman, and they all become fertilized and develop into preimplantational blastocysts. Logically, all these embryos should belong to the donating couple, whose prerogative it would be to determine their fate. The couple might decide that all the embryos must be given the opportunity to implant, that any extras might be given to a woman desirous of pregnancy but with no ovaries of her own, that they might be used for research or that they should be washed down the sink.

A clinical application closely allied to the relief of tubal infertility is the relief of ovarian infertility. In this circumstance, a woman, induced by love or money, would donate an ovum to be fertilized *in vitro* by the sperm of a man whose wife lacked ovaries but had a normal uterus. The

resulting embryo would then be transferred to the uterus of this woman, who would carry the fetus to birth. Such a procedure should be no more objectionable than artificial insemination.

A more controversial variation involves the issue of surrogate uteri, or, as it has been termed, "rent-a-wombs." Here, the oocyte would be fertilized by the sperm of the husband of the egg donor, and the embryo would then be transferred to the uterus of another woman whose menstrual cycle had been properly timed. This woman might receive a fee to carry the pregnancy to term. Presumably, the donor of the oocyte would not want to bear the pregnancy because of health reasons, job obligations, fear or vanity. Much has been made of the possibility of "exploitation of the poor" by such a scheme. However, the other side of the coin is represented by a few women who have called our office (though we are not engaged in work of this nature) to inquire whether they might volunteer their services should such ventures become reality. They state that they love being pregnant, and would arrange to always be in this condition if it were not for the matter of having to keep the babies. They think that hiring out their uteri would be a fine way to make a living. On reflection, it seems inconsistent to categorically deny such women this kind of livelihood, while we permit and even encourage people to earn money by such dangerous means as coal mining, or racing little cars around a track at 200 miles per hour. Perhaps the genuine major concern in the application of this scheme is the possibility that the woman attached to the rented womb might become psychologically attached to her tenant, and not wish to relinquish possession at birth. Or, conversely, that should the baby be born abnormal, the "donor parents" might refuse to accept the child.

Another possible application of preimplantational ectogenesis relates to the work of Gardner and Edwards, who were able to accurately determine the sex of preimplantation rabbit embryos by determining the sex chromatin in biopsied fragments of the trophoblastic portions.[92] Should this work prove possible in human embryos, it would provide an alternative for determination of sex of offspring. Such preimplantational sex determination would be highly preferable to the only method of sex choice now available: amniocentesis with abortion of embryos of the undesired sex. The technique would be welcomed by couples for use in routine family planning, as well as by women and men who carry the genes for X-linked disorders like hemophilia and Duchenne muscular dystrophy.

At some time in the future this technique of embryo biopsy might be extended considerably. Karyotyping of the cells would provide a method of screening for chromosomal diseases, such as Down's syndrome. Autosomal recessive disorders which manifest as inborn errors of metabolism secondary to enzyme deficiencies might be detected by microchemical assays. Perhaps even multifactorially-caused birth defects such as neural tube defects or cleft lip and palate may eventually be found to be associated with a characteristic biochemical abnormality in the preimplantation embryo. Therefore, one day, *in vitro* fertilization and embryo culture could become the preferred mode of reproduction, with transfer to the uterus of only genetically-healthy embryos. In this way, considerable inroads could be made in the prevention of many all-to-common serious birth defects.

Propositions of this sort invariably elicit cries of "Brave New World," and fears of governmental control of reproduction, complete with compulsory "elimination of the unfit."[93] This, however, could only happen if a government were to obtain the power to regulate reproduction in the first place. Reproductive control would necessarily be the result, not the cause, of slavery. And if a government ever should be able to wield such power, it is likely that reproductive control will be the least of our worries. Far more likely to become reality is the concern that the more militant members of Right-to-Life groups will succeed in legislating *their* brand of morality, thereby forbidding individual couples to elect these particular reproductive techniques.

Eventually, manipulation of preimplantational embryos might also be used in genetic engineering, to permit replacement of a defective gene by a normal one. This could be done by exposure of the embryo to a virus containing the properly-functioning gene needed by the embryo: the virus would integrate itself into the chromosomes of the embryo, becoming, in fact, a basic part of the genotype of the new organism.[94] Alternatively, the problem might be solved by injection of a number of cells from a genetically-competent embryo into the blastocele cavity of the deficient embryo. In this fashion, a chimera would be formed.[95]

In summary, the foundations of civilization

probably would not tremble if the gametes of some men and women were to make union in plastic tubes rather than in fallopian tubes. But before this becomes an accepted practice, it would seem preferable, as suggested by Lappé, to gather considerably more animal data, in order to better determine the safety factor. Just how significant and representative were Chang's microphthalmic rabbits?

In any event, it seems premature to recommend the use of preimplantational ectogenesis in humans. Aside from the issue of safety, the technology simply is not sufficiently mature. Edwards' two published photographs of blastocysts appeared normal, and so have the photographically-reproduced embryos cultured by some other groups of scientists. But still, we have no definitive evidence that *in vitro* fertilization in humans has yet been accomplished. No Y chromosomes have been identified in these embryos. Moreover, there still has been no published report providing the details of last year's sensational verbal claim by D. C. A. Bevis that he had produced three children in 36 trials of *in vitro* fertilization and transfer.[96] Perhaps the best evidence that preimplantational ectogenesis is not likely to become routine in humans in the near future is provided by the work of Mukherjee in mice. In this species, where oocytes are plentiful, easily obtained and easily transferred in a sterile manner, only between one and two oocytes in every 100 were able to complete total preimplantational ectogenetic development, and then develop to term after transfer. This is probably just as well: in the time it will take to improve the technology and determine the safety of preimplantational ectogenesis, society will have the opportunity to slowly assimilate the concept and adjust its attitudes with regard to it.

Preimplantational Ectogenesis in Medical Research

Even if no human beings ever result from preimplantational ectogenesis, the procedures still hold considerable potential for amelioration of human problems via basic research.[97-100] The most obvious set of applications in this regard would center around investigations of peri-fertilization events. Many important aspects of chromosomal behavior at this crucial time would be amenable to investigation: for example, normal and abnormal chromosomal disjunction, chiasma frequencies and mechanisms of terminalization, and the behavior during meiosis and cleavage of both balanced and unbalanced translocations. Study of gametes and early embryos might provide clues important to the solving of hitherto-unexplained cases of habitual abortion and infertility. On the other hand, a better understanding of fertilization, including the cortical reaction and capacitation, might lead to the development of more effective and safer contraceptives. Investigations concerned with the activation of new enzyme systems in the developing embryo would be important to our knowledge of control of gene activity during development; closely related to this concept would be studies of the inactivation of the X chromosome in female embryos, and investigations having to do with cellular differentiation and dedifferentiation, so important in cancer research. The concept of keeping embryos in the deep-freeze would be (and in fact is) of great fascination to both conservationists interested in new ways of safeguarding the survival of endangered species, and commercial animal breeders interested in new ways of increasing profits.

Conclusion

Preimplantational ectogenesis offers considerable potential for improving the human condition. However, the field is in its infancy, and too much should be neither expected nor attempted too soon. A properly slow and critical approach should both guard against unfortunate results and give society the opportunity to adjust and reshape some basic philosophical concepts.

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